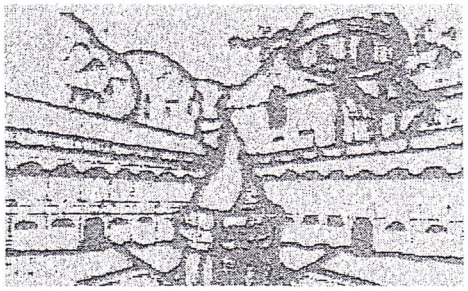
8ª REUNIÓN DE LA RED ESPAÑOLA DE LEVADURAS



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Fission yeast Rho-GEF Rgf1p links morphogenesis and the DNA replication checkpoint.

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Our lab study polarized growth in fission yeast and is focused on understanding the role of Rho1p and its GEFs -Rgf1p, Rgf2p and Rgf3p- in cell morphogenesis. Rho1p is the regulatory subunit of the β–glucan synthase (GS) and is involved in actin organization, exocytosis and stress responses. To coordinate all these processes Rho1p must be at the hub of many signalling pathways and therefore it should be tightly regulated. Previous work from our laboratory (in fission yeast) and from others, demonstrate that a particular GEF regulates a subset of Rho1p functions, specifically linking the stimulus induced signalling to a particular response.

Rgf1p is a multi-domain protein involved in different macromolecular processes. Rgf1p localises to cortical sites at the cell ends, from where this protein activates the β-GS subunit Bgs4p and signals upstream from the Pck2p-Pmk1p MAPK signaling pathway after (Garcia et al, MBC, 2009). Moreover, Rgf1p is also necessary for the establishment of bipolar growth also called NETO (New End Take Off). While extensively study, it is currently unknown how the cell cycle machinery regulates this transition and how the actin cytoskeleton and the cell wall are remodelled in response to the spatial distribution of the microtubules.

As a first step toward analyzing the regulation of Rgf1p, we study the localization of mutated versions of the Rgf1p-GFP. Unexpectedly, we found that mutants in the Rgf1p DEP domain (*Dishevelled*, *Egl-10*, and *Pleckstrin*) localized to the nucleus and that this domain was nearby a canonical nuclear localization signal (NLS). We have identified a region of 200 aa at the N-terminus of Rgf1p-GFP necessary and sufficient for nuclear localization. Wild-type Rgf1p-GFP also localizes to the nucleus in Hydroxyurea (HU) treated cells and nuclear localization was dependent on the 14-3-3 protein Rad24p and on the Cds1p (the DNA replication checkpoint kinase). Moreover, *rgf1*Δ cells were sensitive to HU on a plate assay. Our data are consistent with a temporal and spatial regulation of Rgf1p during the cell cycle and will allow us to investigate the mechanisms linking the completion of DNA replication with a growth polarity transition.